

**CLAIMS**

1. Isolated photoprotein containing an amino acid sequence which:
  - a) is able to bind coelenterazine and calcium, producing  
5 bioluminescence;
  - b) is identical by at least 90% to SEQ ID NO: 1 (Clytin);
  - c) in sequence alignment with SEQ ID NO: 1 (Clytin), presents one of the following single or multiple substitutions (the residue positions are referred to SEQ ID NO: 1):
    - 10 i) C<sub>54</sub>→S;
    - ii) S<sub>132</sub>→C;
    - iii) K<sub>48</sub>→R, N<sub>195</sub>→D;
    - iv) Q<sub>68</sub>→R, A<sub>90</sub>→V, T<sub>184</sub>→I;
    - v) Y<sub>82</sub>→F, K<sub>110</sub>→N, F<sub>125</sub>→L, S<sub>149</sub>→R;
    - 15 vi) G<sub>142</sub>→C;
    - vii) I<sub>53</sub>→V, S<sub>149</sub>→R;
    - viii) N<sub>18</sub>→D, I<sub>40</sub>→V, K<sub>56</sub>→R;
    - ix) Gly<sub>58</sub>→Glu, Asp<sub>69</sub>→Val, Ala<sub>70</sub>→Cys, Lys<sub>76</sub>→Arg, Lys<sub>77</sub>→Gly, Ile<sub>78</sub>→Cys, Asp<sub>81</sub>→Glu, Val<sub>86</sub>→Ile, Glu<sub>87</sub>→Ala, Ala<sub>90</sub>→Gln, Val<sub>92</sub>→Leu, and Glu<sub>97</sub>→Gln  
20 a functional derivative or fragment thereof.
2. The photoprotein of claim 1, containing an amino acid sequence identical by at least 95% to SEQ ID NO: 1.
3. The photoprotein of claim 2, containing an amino acid sequence  
25 identical by at least 98% to SEQ ID NO: 1.
4. The photoprotein of claim 3, containing an amino acid sequence which is selected from the group consisting of SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10.
5. A photoprotein according to claims 1-4, wherein said amino acid

sequence is fused to a mitochondrial target sequence.

6. An isolated polynucleotide encoding a photoprotein according to claims 1-5.
7. The polynucleotide of claim 6, having the sequence of SEQ ID NO: 11,  
5 12, 13, 14, 15, 16, 17, 18, 19.
8. An expression vector containing a polynucleotide according to anyone of claims 6-7.
9. A prokaryotic or eukaryotic host cell containing the vector of claim 8.
10. A mammalian host cell according to claim 9.
- 10 11. A method in vitro for detecting changes in intracellular calcium concentration which comprises:
  - a) providing a cell expressing a photoprotein according to claims 1-5;
  - b) contacting the cell with an agent stimulating calcium influx or calcium release from intracellular stores;
  - 15 c) detecting the photoprotein bioluminescence.
12. A method of screening compounds modulating intracellular calcium concentration, which comprises:
  - a) providing a cell expressing a photoprotein of claims 1-5;
  - b) contacting the cell with the candidate compound;
  - 20 c) detecting the bioluminescence of the photoprotein.
13. A method according to claims 11 or 12, which is carried out in a high-throughput format.
14. A method according to claim 13, which is carried out with a high throughput optical screening apparatus suited for multi-sample analysis.
- 25 15. The use of a photoprotein according to claims 1-5 as intracellular calcium indicator.
16. The use of a photoprotein according to claim 15 in a cell-based high throughput assay.

17. The use of a photoprotein according to claims 1-5 for the preparation of a diagnostic composition.